## REMARKS

Applicants have amended claim1 in order to more particularly define the invention taking into consideration the outstanding Official Action. If this amendment does not place the application in condition for allowance, the undersigned attorney would like to conduct an interview with the Examiner in an effort to resolve any outstanding issues. Applicants believe that the presently claimed invention is clearly patentable over the references of record and the claims are in full compliance with 35 USC 112. In an effort to clarify the claimed invention, Applicants submit herewith further figures which schematically illustrate the embodiments of the claimed invention.

Figure 1 as enclosed is an amended version of that submitted with Applicants previous response and is a schematic diagram of the relationship between cobalamin, TCII and HC. As the Examiner will appreciate, the terms TCII (Transcobalamin II) and HC (Haptocorrin) are used to indicate two classes of cobalamin binding proteins with or without cobalamin bound. The protein without bound cobalamin is referred to as "apo-" and with cobalamin in place is termed "holo-". Only a small proportion of the cobalamin in a body sample is in the form of holo-TCII. The remainder is in the form of apo-HC. The total cobalamin level is the sum of the cobalamin bound in holo-TCII and holo-HC.

These various components are now shown explicitly in Figure 1. Specifically, those parts containing cobalamin are shaded with diagonal hatching, those parts comprising HC have vertical stripes and those comprising TCII have horizontal stripes. The component of interest in the current invention is cobalamin bound to TCII (ie holo-TCII), which is shown as the arc with both horizontal and diagonal shading.

Figure 2 uses the same components shown in Figure 1 to illustrate various embodiments of the invention in which binders for subsets of the sample are used to separate out the holo-TCII. This process is carried out experimentally by binding one component of a mixture to an immobilised or immobilizable ligand, separating this fraction from the unbound fraction and then isolating the desired component from either the bound fraction (by releasing the bound components) or the unbound fraction (by

removing the ligand and bound components). In the embodiment shown by the left-hand path, TCII is bound and washed free of HC. When the TCII is released from the ligand, the only cobalamin remaining is that which is contained as holo-TCII. This can then be separated from the apo-TCII if desired. The central path shows the effect of a binding ligand specific for holo-TCII. This allows all other components to be washed away leaving only the component of interest. The right-hand path shows an alternative embodiment in which apo-HC and apo-TCII are initially bound (eg by a cobalamin analogue) and this binding is used to remove these components. A second specific ligand is then required to fractionate the remaining holo-TCII from holo-HC.

It will be appreciated that in each case, at least one step is required in which a specific binding ligand for TCII or holo-TCII is employed. This is a crucial step which allows the function of the claimed invention by both separating and concentrating the holo-TCII.

The separation and concentration effect is demonstrated in the embodiment shown in Figure 3. This shows the addition of a TCII binding ligand to a sample containing TCII and HC. By binding and removing the TCII (holo- and apo-) the ligand separates this from the HC. The bound TCII can then be **released into a reduced volume** thereby also concentrating the sample. Without this concentration effect it is impossible to quantify holo-TCII with standard assays as it is simply too dilute. The ratio of the final concentration to the original sample concentration relies on the binding efficiency and the respective volumes in the original sample and the release medium. All of the enclosed figures are based on embodiments of the invention described in the original disclosure.

Taking this background into consideration, the points in the Official Action will now be discussed seriatim.

The rejection of the claims under 35 USC 112, second paragraph as set forth in item 3 of the Official Action has been carefully considered but is most respectfully traversed. The Examiner states that claim 1 is vague and indefinite because of an apparent inconsistency between the quantity to be determined and that measured. Applicants most respectfully submit that the reason that "transcobalamin II bound

cobalamin" in the first line is equivalent to the measurement of "cobalamin content" in the final line is that the intervening steps remove other sources of cobalamin leaving only the cobalamin that was bound to TCII for analysis. An example of this effect is illustrated in Figure 3, by which it can be seen that the cobalamin arising in the final liquid is only that which was present bound to TCII in the original sample.

Obviously, since it is the holo-TCII level that is to be assayed, this can be measured in the final step either by analysis of the cobalamin content of the holo-TCII or by measuring its TCII protein concentration. This has been clarified in enclosed claim 1 which recites "by measuring a cobalamin or a TCII-protein content arising from the holo-TCII released from the specific binding ligand".

For consistency and in order to clarify that the assay is for determining the concentration of the complex of cobalamin bound to the TCII-protein, the term holo-TCII has been used throughout the claim.

The rejection of claim 1 is also on the basis that "There is no correlation step which correlates the determining the cobalamin content to the determination of the transcobalamin II bound cobalamin". However, Applicants most respectfully submit that this is the exact feature which is specified in the middle portion of the claim as would be appreciated by one of ordinary skill in the art to which the invention pertains. By contacting the sample with a ligand for TCII and then separating the bound and non-bound portions, the content of cobalamin is reduced to only that which was bound to the TCII-protein (ie as holo-TCII). This is absolutely inherent in the method described but has been stated explicitly in amended claim 1 to increase the clarity of the claim.

The Examiner states that it is "unclear whether the released cobalamin concentration is being assayed or if the cobalamin bound by TCII is being assayed". Since, after the specific binding and release steps, the cobalamin present in the cobalamin containing liquid is only that which was present in the sample as holo-TCII, these two quantities are exactly the same. It therefore makes no difference which is measured and the holo-TCII or cobalamin in this sample may be measured interchangeably but the value of the cobalamin is determined in either case because of this equivalency. Applicants refer again to enclosed Figure 3.

The rejection also refers to the method of concentration of the sample and specifically states "It is unclear how to determine how much at least 3 times the amount of cobalamin is ... if the amount of holo-TCII is unknown. As illustrated in Figure 3, the degree of concentration is controlled by the specificity of the ligand and the relation of the volumes of the cobalamin containing sample and the liquid into which the bound TCII or holo-TCII is released. This is clearly described on lines 5-9 of page 8 of the specification. The degree by which the cobalamin in the sample is concentrated is quite independent of the original concentration and is controlled solely by the binding and the original and final volumes. This feature is absolutely inherent in the claimed method as would be appreciated by one of ordinary skill in the art. However, for increased clarity the passage "into a volume of liquid which is at least 3 times less than the volume of said cell free sample" has been inserted into claim 1. It is absolutely clear to a skilled worker that this decreased volume is directly related to the increase in concentration. Accordingly, it is most respectfully requested that the rejection under 35 USC 112 be withdrawn.

The rejection of claims 1, 5-7 10,12, 16-20, 26 and 42-44, 47-48 and 50 under 35 USC 103(a) as being unpatentable over McLean et al., in view of Houts has been carefully considered. This rejection is traversed for reasons of record, in view of the further amendments to the claims and the following comments.

In the Official Action it is urged that "the claims do not recite only holo-TCII levels are being assayed. The claims broadly recite transcobalamin II which includes TCII and holo-TCII". As previously formulated, the claims in fact related to "transcobalamin II bound cobalamin" which is, of course, holo-TCII. As noted above, the term "holo-TCII" has been employed throughout amended claim 1 in order to avoid any ambiguity on this point. Since claim 1 clearly recites an assay for holo-TCII, the Examiner is requested to reconsider the arguments relating to McLean in view of Houts previously submitted in view of this amendment.

The Official Action also states "McLean in view of Houts teaches an assay method of determining transcobalamin bound cobalamin in a sample". As discussed in the Applicant's previous responses however, McLean relates to a method of testing

antibodies for their potential as anti cancer agents. This is carried out by measuring how effectively the antibodies prevent the proliferation of cells by starving them of the TCII required to transport cobalamin into the cells. This is in no way an assay for holo-TCII and contains absolutely no teaching towards an "assay method for the determination of holo-transcobalamin II" as presently claimed.

Houts (US 4,465,775) was also previously discussed in detail in the Applicant's last submission and also fails to teach an assay for holo-TCII. As one of ordinary skill in the art would appreciate, only one component of holo-TCII and at least 75% of the cobalamin in a sample is bound to HC rather then to TCII (see Figure 1 for a schematic illustration). In order to render obvious the presently claimed invention, it would be necessary to provide a method for assaying holo-TCII by separating the TCII component of a sample form the component non-TCII components (eg HC), concentrating the TCII component by at least 3 times and measuring the resulting holo-TCII level. Each of these aspects is a specific limitation of claim 1 which cannot be ignored and none are either disclosed or suggested in either of Houts or McLean or any combination thereof. The necessary teaching or motivation to arrived at the claimed invention must be found in the prior art and Applicants' specification may not be used as a teaching reference. In re Fritch, 23 USPQ 1780, 1784(Fed Cir. 1992) ("It is impermissible to engage in hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps.). Moreover, obvious to try is not the standard of obviousness under 35 USC 103.

The Examiner notes, essentially correctly, that McLean teaches the role of cobalamin in DNA synthesis. This is, however, of little significance to the currently claimed invention, since it is not cobalamin which it is to be assayed by holo-TCII. The high concentrations of cobalamin which have been supplied to patients with a genetic deficiency in TCII are given because a small amount of cobalamin (around 1% of the amount transported by TCII) can be absorbed by cells through direct diffusion across the cell membrane. Thus, people with very low TCII levels can be saved from acute cobalamin deficiency by abnormally increasing their blood cobalamin levels. This it is, of course, completely unrelated to the concentration step of the currently claimed

invention, since it is the holo-TCII in the sample which it is concentrated, rather than that in the patient themselves. The claimed method is an assay for holo-TCII levels, rather than a method of treating patients.

The applicant's argument that neither Houts nor McLean teach the concentration of a holo-TCII containing sample it is rejected by the Examiner on the basis of McLean page 241 paragraph 3. The increase in concentration applied in McLean it is, however, completely different to that currently claimed. In McLean, an antibody is used to block the transport of cobalamin as holo-TCII. As noted above, however, a small amount of cobalamin can enter a cell unaided and thus by increasing the free cobalamin level dramatically, McLean can rescue cells which will otherwise not grow due to antibody-induced cobalamin starvation. This use of free cobalamin to overcome TCII deficiency is quite distinct from the applicant's invention, which relies on highly specific binding of TCII to separate and concentrate the cobalamin from holo-TCII into a small volume for testing. Reference it is again made to the embodiment represented schematically in Figure 3.

The Examiner rejects the Applicant's arguments on the basis that they address the citations individually, rather than together. It is important to note, however, that **neither of the cited documents teaches an assay method in which the cobalamin present as holo-TCII in a sample it is separated and concentrated for analysis**. The citations, either together or in combination must teach towards this limitation in order to render claim 1 obvious and they do not. The reference in McLean to high concentrations of cobalamin it is in the context of the addition of excess cobalamin. The concentration effect claimed in the current application it is produced as a result of the claimed specific binding and release method. This method of specific binding and release into a reduced volume, so as to provide the existing cobalamin in a smaller volume and thus with a correspondingly high concentration, has no parallel in McLean when combined with Houts.

It is relevant to note that the lower limit of quantification in the standard curve from Houts it is at around 50 ng/L (around 40 pM). This it is the detection limit of a **standard cobalamin assay** and thus these assays **cannot be used to assess the**

**holo-TCII concentration of a patient with borderline holo-TCII deficiency** since the normal range it is 35-160 pM (see page 6 lines 2-5). Thus the techniques of Houts cannot be applied directly to a useful holo-TCII assay since the technique is not sufficiently sensitive. A step which increases the sample concentration by a **known factor** is essential if standard analytical techniques are to be used in determining the very small amount of cobalamin present as holo-TCII. Such a step cannot be deduced from the combination of Houts and McLean as would be appreciated by one of ordinary skill in the art to which the invention pertains. Again, applicants' specification may not be used as a teaching reference.

Applicants use a specific binding separation and concentration method to test for possible cobalamin deficiency by assaying holo-TCII, rather than total cobalamin. If anything, McLean teaches that TCII it is **not** the only way in which cobalamin can enter cells and thus it is not an absolute requirement for cobalamin uptake. It thus teaches away from the development of a holo-TCII assay for the assessment of cobalamin deficiency in a subject. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 9, 11, 24-25 and 35-36 as set forth in item 6 of the Official Action has been carefully considered but is most respectfully traversed for reasons already of record and the amendment to the claims. The teaching of the additional reference does not overcome the deficience of the McLean and Houts references as discussed above. That is, these claims are, however, all dependant upon independent claim 1 which, as noted above, it is by no means obvious in view of the prior art. Claim 25 relates to the binding strength of the specific binding ligand used in the separation and concentration step. By employing a binding ligand that removes 80% of the TCII component from a sample and releases this into a greatly reduced volume, a significant separation and concentration of the holo-TCII is achieved. Again, there is no suggestion in any of the citations that a ligand could be used to extract 80% of the TCII from a sample. Finally, claims 35-36 are extremely stringent limitations. These require, respectively, that a specific binding ligand be used that can remove sufficient TCII from a sample and release it into a sufficiently small volume so as to increase the

concentration by 5 or 10 fold. No such separation and concentration effect is indicated in any of the cited documents. The Applicant notes however that claim 3 recites a holo-TCII detection limit of 9 pM, which it is at least 3 times lower than the lower quantification limit of a standard cobalamin assay. It is therefore a very significant limitation which cannot be ignored. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 4 and 49 under 35 USC 103(a) as being unpatentable over Mclean et al. in view of Houts in further view of Allen et al. has been carefully considered but is most respectfully traversed. The claims relating to automation (4 and 49) are rejected on the basis that the Examiner considers automation to be an obvious variant to one with ordinary skill. The Applicant notes however that it is only obvious to apply automation to a technique that can be carried out by automated methods. The assay described in Houts cannot be applied to holo-TCII because it lacks the necessary sensitivity. There can therefore be no question of automating this for the detection of holo-TCII. A skilled worker would be aware that previous assays capable of detecting holo-TCII at biological concentrations were highly time consuming and inappropriate for automation (see page 6, first full paragraph). It is thus only obvious to automate the assays of the current invention once an assay has been established which it is highly sensitive and suitable for automation. None of the cited references provide teaching towards such an assay. Again, Applicants specification may not be used as a teaching reference and "obvious to try" is not the standard of obviousness under 35 USC 103(a). Accordingly, it is most respectfully requested that this rejection be withdrawn.

Finally, the rejection of claims 27-33 relating to the specific binding ligand as obvious in light of McLean, Houts and Hoyle has been carefully considered but is most respectfully traversed for the reasons discussed previously and above with respect to the teachings of the McLean and Houts references. The teachings of Hoyle do not overcome the deficiencies of the primary references. It remains the case however that the use of specific binding ligands with the claimed binding affinity and specificity in separating and concentrating TCII is never indicated and it is most respectfully requested that this rejection be withdrawn.

Applicants submitted with the amendment filed on January 29, 2001,the necessary certified copy of the priority document to complete the claim for priority. An acknowledgment of the claim for priority and receipt of the priority document in the next Official Action is most respectfully requested.

In view of the above comments and further amendments to the claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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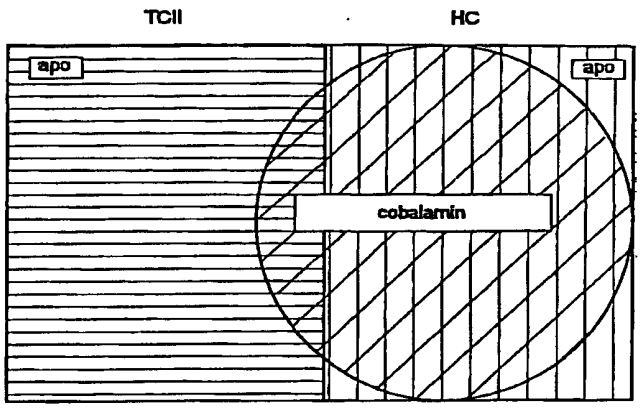
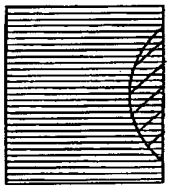
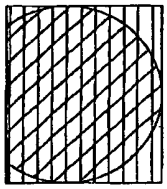
## MARKED-UP VERSION OF CLAIMS

1(Thrice amended). An assay method for the determination of [transcobalamin II bound cobalamin] holo-transcobalamin II (holo-TC II)in a body sample, comprising contacting a cell free sample of a body fluid with an immobilized or immobilizable specific binding ligand for transcobalamin II (TC II) or holo-transcobalamin II (holo-TC II), separating a ligand bound fraction from a non-ligand bound fraction, releasing cobalamin in the ligand bound fraction into a volume of liquid which is at least 3 times less than the volume of said cell free sample, to provide a cobalamin containing liquid wherein the cobalamin concentration is at least 3 times the holo-TC II concentration in said cell free sample of body fluid and determining the cobalamin content in said cobalamin containing liquid by measuring the cobalamin or TCII-protein content arising from the holo-TCII released from the specific binding ligand.

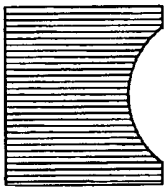


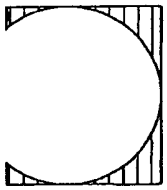
TCII (Transcobalamin II)



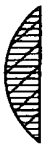
HC (Haptocorrin)



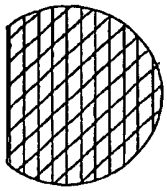
apo-TCII (TCII with no cobalamin)



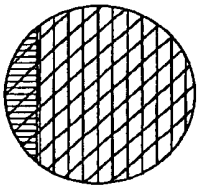
apo-HC (HC with no cobalamin)



holo-TCII (TCII with cobalamin bound)
(Biologically functional cobalamin)



holo-HC (HC with cobalamin bound)



Total cobalamin (= holo-TCII +holo-HC)

Figure 1

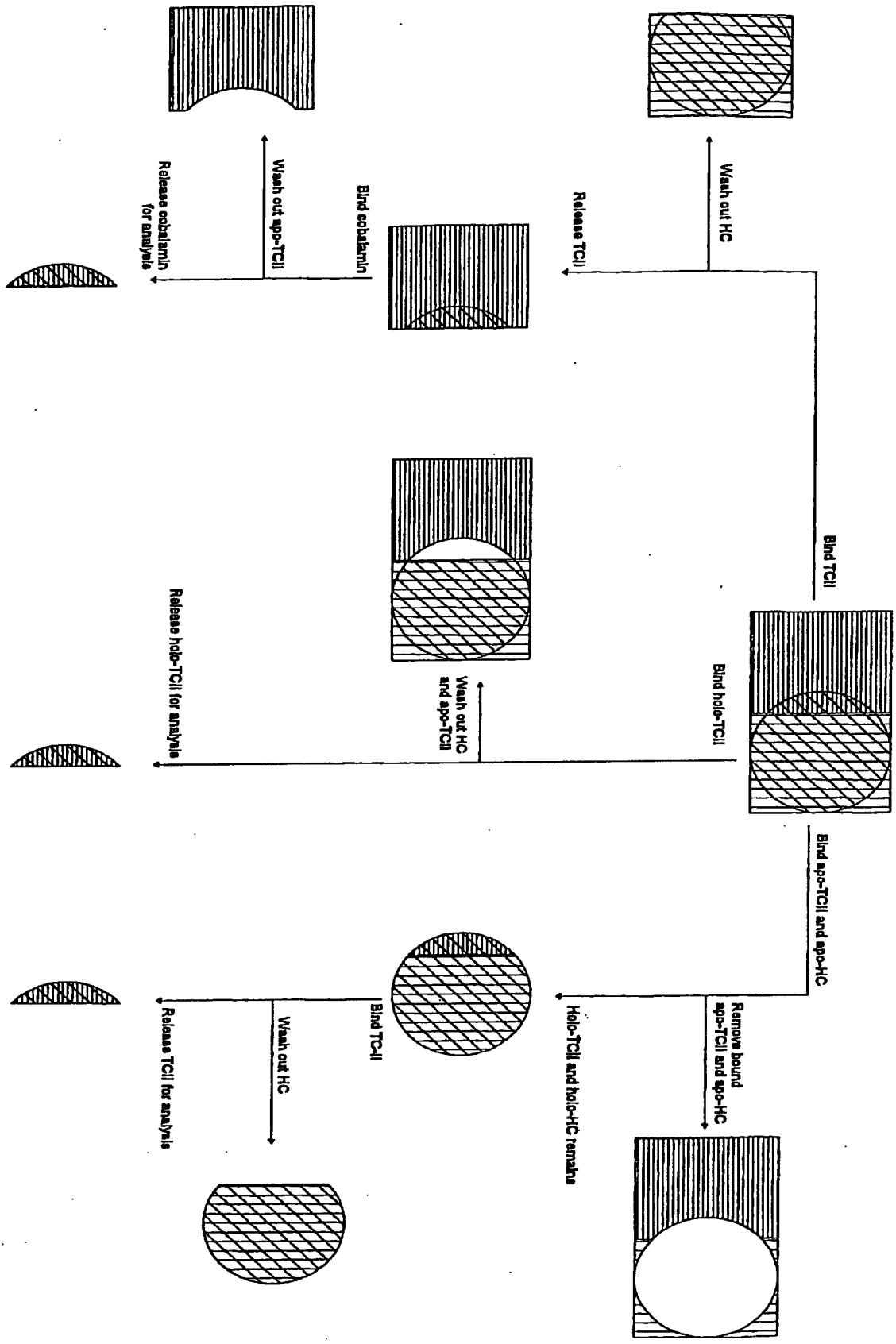

Wash out HC
Release TCII
Bind TCII
Bind holo-TCII
Bind apo-TCII and apo-HC
Remove bound apo-TCII and apo-HC
Holo-TCII and holo-HC remains
Bind TC-II
Wash out HC
Release TCII for analysis
Wash out HC and apo-TCII
Release holo-TCII for analysis
Bind cobalamin
Wash out apo-TCII
Release cobalamin for analysis

Figure 2

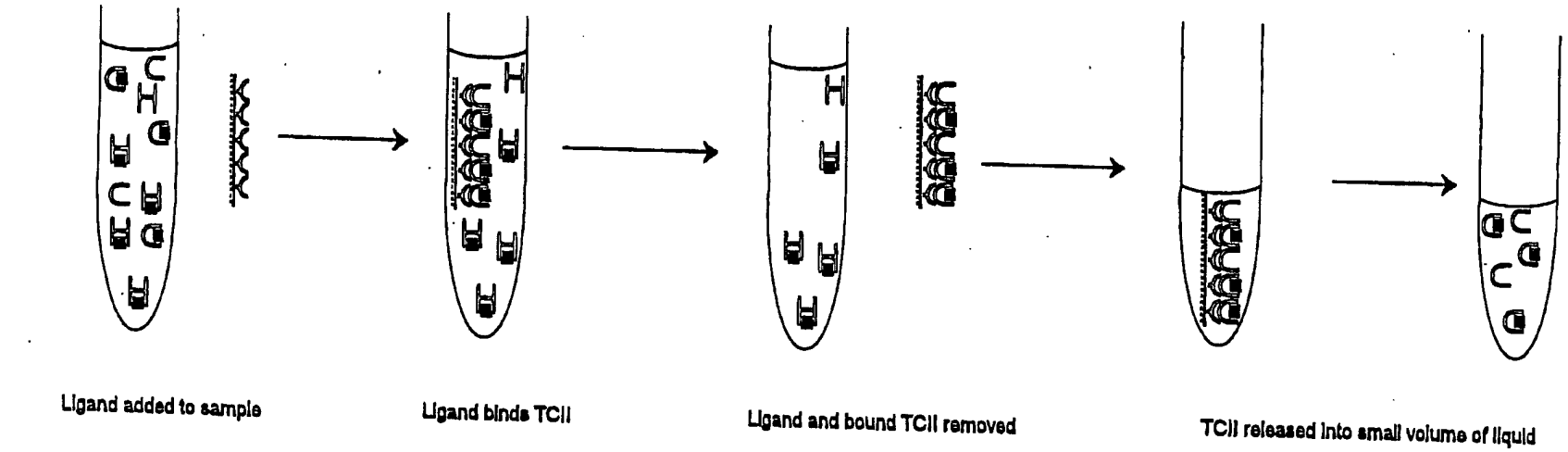



Figure 3